Effect of including double bunks and straw on behaviour, stress response production performance and meat quality in feedlot lambs

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A B S T R A C T

The development of proper environmental enrichment would be a useful tool to tackle the welfare problems associated with intensive indoor lamb production. This study analyses the effect of enrichment with double bunk and cereal straw on behaviour, use of space, stress response, productive traits and meat quality variables during the finishing phase of fattening lambs. Sixty lambs were placed in enriched (ED) or conventional (CO) pens (3 pens each treatment, 10 lambs/pen) where ED had a double bunk with ramps and cereal straw as bedding and forage. The CO pen was barren, similar to commercial feedlots, without straw or any other enrichment. The proposed ED had a positive effect on the behavioural characteristics of the lambs, which displayed a richer behavioural repertoire, fewer stereotypes and more affiliative interactions, improving group cohesion. Moreover, ED lambs had enhanced immunity and there were no discernible detrimental effects on performance or on the commercial quality of their meat. However, the enriched system proposed may pose difficulties for feedlot handling procedures.

1. Introduction

As in several other Mediterranean countries, in Spain the traditional pastoral sheep production system is changing towards more intensive schemes with large flocks and increased productivity (de Rancourt et al., 2006; Miranda-de la Lama et al., 2010a). The creation of temporary lamb feedlots or classification centres (CC) externalizes the final fattening stage, outsourcing it to an off-farm unit, which simplifies the process for the farmer and improves carcass homogeneity (Miranda-de la Lama et al., 2009). However, this practice has caused new problems, such as dependency on an external resource, multiple live transports, social mixing and frequent handling in uninspiring environments (Aguayo-Ulloa et al., 2013; Miranda-de la Lama et al., 2010b). Previous studies have suggested that factors associated with handling during transport, regrouping of individuals, feeding and novel environments of feedlots may cause significant stress (Miranda-de la Lama et al., 2010b), increasing susceptibility to multifactorial disease and affecting behaviour and physiology (Miranda-de la Lama et al., 2012). These studies have suggested that level of aggression, stereotypes and stress physiology variables are useful for highlighting welfare problems in feedlots.

Possibly due small ruminant systems are perceived, generally, by consumers to be associated with a high standard of welfare, especially in relation to aspects of naturalness, research has not always focused on this sector, as happened with the concluded EU-funded Welfare Quality® project, that did not include small ruminants in its remit (Goddard, 2013). It is necessary, however, to establish specifics protocols that are able to minimize the biological cost of the adaptation of animals to a novel feedlot environment, which in part, it has recently been working on the AWIN (www.animal-welfare-indicators.net/site/), another EU-funded project that includes small ruminants. The development of proper environmental enrichment would be a useful tool to tackle welfare problems associated with intensive indoor production systems. However, to date there has been little data available on the effect of environmental enrichment on behaviour, stress physiology, productive traits and meat quality of indoor fattened lambs.

Studies with a modified housing environment for sheep using different floor levels were mainly focused on availability of space in relation to ecological production systems (Hansen and Lind, 2008).

Our study is based on the hypothesis that enrichment during the finishing period of fattening may improve the adaptation process of the lambs to the feedlot environment which, in turn, may optimize their welfare and performance. The goal is to analyse the effect of functional full enrichment (Aguayo-Ulloa et al., 2014) on a combined set of variables. The effect of the enrichment with double bunk and cereal straw were measured in terms of behaviour, stress response, productive traits and meat quality variables during the finishing phase of fattening lambs

2. Methods

The study was carried out using facilities at the Animal Experimentation Service of the University of Zaragoza in the Autonomous Community of Aragon, Spain (41°41′ N). All the lambs were raised, transported and slaughtered according to the current regulations of the European Community Commission (1986) for Scientific Procedure Establishments. Experimental protocols were approved by the Animal Experimentation Ethics Committee of the University of Zaragoza (ES 50 297 0012 006).

2.1. Study description

Sixty *Rasa Aragonesa* entire male lambs (65 days old) with an average live weight of 17.1 (±0.19) kg, were allocated to two treatments (weights were balanced across treatments) according to their pen environment during the finishing phase of fattening, which lasted five weeks (35 days). Lambs were housed indoors in six pens with 10 lambs each (2.9 × 3.3 m, 0.95 m^2 per lamb) and three replicates per treatment. Lambs from the enriched group (ED) were kept in pens with a wooden double bunk with two ramps that provided access to the upper part of the platform (Fig. 1). The platform was 2.0 m long × 0.95 m wide × 0.5 m high. The double bunk was attached at the corner of the solid fence separating each pen, allowing the lambs to explore, to lie down (on the upper or ground level of the double bunk), to play and/or to seek shelter. The ED lambs were provided with cereal straw as bedding on the floor and as forage in a fodder rake. Lambs from the control group (CO) were kept in a barren pen, without any enrichment (mimicking the feedlot environment). As CO lambs did not have any straw, for hygienic reasons a thin layer of sawdust was added at the beginning of the experiment. All lambs were fed with commercial concentrate (Ovirum Alta Energía®) containing barley, wheat, calcium carbonate, sodium chloride and a vitamin supplement corrector (18% crude protein and 11.5 MJ metabolisable energy/kg DM). Feeding and water consumption were ad libitum. In both treatments the concentrate hopper was wide enough to allow all lambs to eat simultaneously. Water was provided using a float drinker installed in a corner of each pen. Feed consumption (concentrate) was recorded to estimate the conversion index during the fattening period. Animals were weighed individually at the beginning of the experimental period (W1) and just before slaughter (W2).

2.2. Behaviour and cognitive test

All lambs were individually identified by numbers or letters painted on their sides and rump with washable spray for animal marking. A video-recording device (model VDVR-9, Circontrol S.A., Terrassa, Spain) was set up in a room close to the pens to record animal maintenance behaviour, use of space, stereotypes and social behaviour. One camera was placed in front of each pen, 2.2 m above to the ground. The video-recordings were made for 12 h/day during the 4th week, between day 21 and 27 (both included) of the fattening period when animals were fully accustomed to the enrichments. All videos were watched by the same trained observer. Maintenance behaviour was recorded at group level, because the behavioural pattern of a single lamb within a group is dependent of the other lambs' behaviour (Hansen and Lind, 2008). However, in our study lambs were individually marked to record social behaviour.

Two kinds of sampling were carried out: instantaneous, every 10 min (8 am to 8 pm for 7 days) with a total of 1512 scan samples per treatment. Maintenance behaviour, stereotypes and use of the space were recorded for each sample. The behaviours recorded included resting (RT, lamb lying down), standing (ST, lamb standing on all 4 legs), walking (WK, lamb on all 4 legs and in motion), feeding on concentrate (FC, lamb searching for feed concentrate in the hopper and eating it), foraging straw (FS, lamb searching for forage straw in the fodder rake and eating it), drinking (DK, lamb drinking water from the drinker), and stereotypes (frequent and non-functional oral manipulation of objects, an abnormal behaviour commonly seen in ungulates in stressful situations, Mason et al., 2007). Recording of stereotypes by scan sampling provided only a general approximation, allowing us to identify the time of day when these were more common. To measure use of space (placement), four areas were defined: the double bunk area (DBA, lambs had at least half of their body on the upper or lower part of the double bunk); concentrate hopper area (CHA, lambs heads were inside the feed hopper or resting with at least half of their body under the hopper); straw rake area (SRA, lambs were foraging or resting with at least half their body in the zone under the rake) and remaining area (RMA, lambs in the rest of the pen). The use of space in CO pens was recorded, tracing the same (albeit imaginary) divisions of the areas in ED pens. Continuous sampling was used to record social behaviour, i.e. the number of agonistic (aggressive) and affiliative interactions per animal (Table 1), and stereotypes, i.e. the number of times that an animal repeated an abnormal behaviour, if perceived as such (as defined by Mason et al., 2007). The scan sampling was used to detect considerable behavioural changes during the course of the day.

The lambs were subjected to a T-maze cognitive test during the last week of the fattening period, in two consecutive rounds. The structure and protocols used were following that described by Aguayo-Ulloa et al. (2014) and using 1.40 m-high plastic panels (Fig. 2). The maze had five areas (three chambers and two corridors) with a start box (0.8 m × 0.5 m) and an isolation chamber joined on one of its sides to a T-corridor (B and C areas). The start box was fully closed but large enough to enable an animal to move around. The T-corridor consisted of a 2 m × 0.80 m path linked to two perpendicular arms (1 m × 0.8 m each) that connected with two chambers (D and E). A mirror (70 cm × 30 cm) and loudspeaker were located in the target zone on the left arm. An observation site was placed on a 3 m high platform, adjacent to the T-maze apparatus, so as not to influence animal movements. The apparatus was kept in a soundproof room (9 m × 6 m) at constant temperature and humidity during the trial.

The sounds used in the experiment were a playback of callings from congeners. The stimulation sound was a computer random selection of bleating of lambs from all pens (same breed, sex and age). More details of recording sounds can be found in Aguayo-Ulloa et al. (2014).

Each lamb underwent the cognitive test during the last week of the experiment, on two consecutive days, once each day of the test, without receiving prior training. Each animal stayed in the start box for 10 s before a guillotine door was lifted to allow it to enter the maze. After the lambs had left the start box, the guillotine door was quietly closed. At the same time, the recording was played to begin the test. The test was successfully passed if the individual found the

Fig. 1. Enriched pen, with double bunk and cereal straw as forage and bedding (above). Control pen, without double bunk and cereal straw (below).

Table 1
Social behaviours recorded during the observations.

Aggressions	Affiliations
Head-butting: when a lamb used the front of its head to make contact with another lamb.	Licking: when a lamb passed his tongue several times over the body of other individual
Body bumping: when a lamb pushed another lamb with its body.	Sniffing: when a lamb sniffed another lamb's body.
Pawing: when a lamb, used its foreleg, to kick other lambs.	Grooming: when a lamb groomed another lamb several times using its teeth.
Chasing: when a lamb actively moved towards another individual, causing the latter to walk or run away.	Following: when a lamb followed another lamb with the intention of keeping close to it.
Mounting: when a lamb mounted another with the intention of moving it away	Sexual mounting: when a lamb mounted another lamb in play or with sexual intent
Biting: when a lamb bit another using its teeth.	Rubbing: when a lamb was rubbed by the body of another lamb.
Threatening: when one lamb threatened another with a head thrust, but without actually making contact	

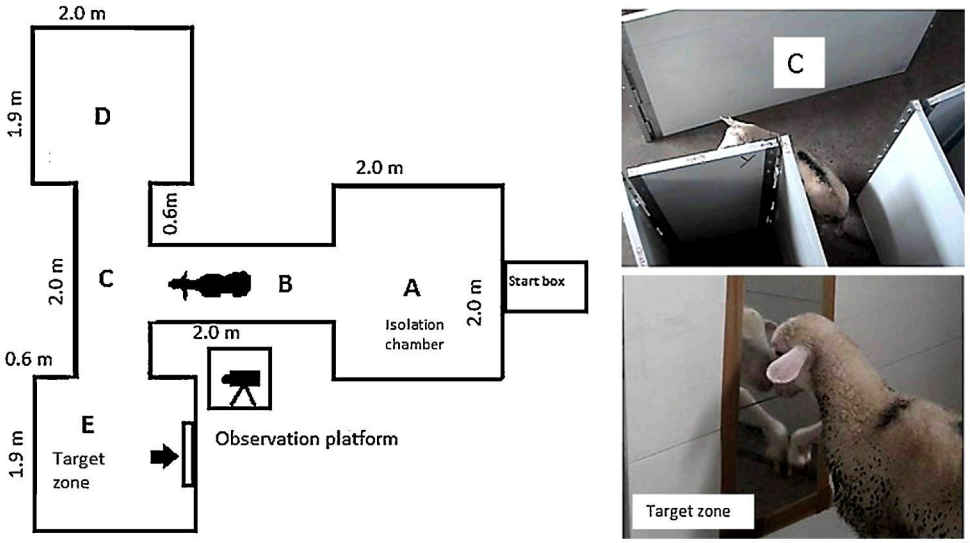


Fig. 2. T-maze apparatus, all dimensions are in metres. The areas passed through were A: isolation chamber; B and C: the T corridor; D and E: chambers. Target zone on chamber E: mirror and source of sound.

target zone (which was always located in the left arm) where there was a social clue: visual source (mirror) and sound source (bleating sound from loudspeaker). Each animal was given a maximum time of 5 min to solve the T-corridor (Aguayo-Ulloa et al., 2014). If an animal did not solve the challenge, it was assigned the maximum time. Each test was filmed, and the time taken by each lamb to solve the T-corridor was recorded. For each lamb and exposure we recorded the total time taken to solve the test, the time that the lamb spent in the first chamber (isolation time) and the number of areas passed through (NQUAD) during the assay (as an estimation of locomotor behaviour).

2.3. Physiological welfare indicators

Blood samples were taken by jugular venipuncture with vacuum tubes (before final weighing) to evaluate physiological responses to stress (two 4 ml tubes per animal, with and without K3-EDTA anticoagulant). Samples were collected by trained personnel that had handled the animals and the venipuncture was carried out as quickly as possible per lamb, as a necessary precaution to avoid sampling error. Samples were kept on ice for a maximum of 2 h and taken to the laboratory for routine haematological measurements. The EDTA plasma and serum were centrifuged at 3000 × rpm for 10 min and aliquots were frozen and kept at −30 °C until analysed. An automatic particle counter (Microcell counter F-800 and auto dilutor AD-260, both from Sysmex™) was used to count red blood cells (RBC) and white blood cells (WBC) (number per litre), haemoglobin (g/dl) and haematocrit (%). The differential leukocyte count was estimated from blood swabs on clean slides. Staining was performed by the rapid panoptic method using dyes from Química Clinica Aplicada Inc. (QCA). Using an optic immersion microscope we counted and identified 100 leucocytes per sample (neutrophils, lymphocytes, eosinophils, basophils and monocytes). The neutrophil/lymphocyte ratio (N/L) was used an indicator of chronic stress (Lawrence and Rushen, 1993). Serum samples were used to determine the concentration of glucose (mg/dl, Ref. Glucose AE2-17), and the activity of creatinine kinase (CK) (UI/L) (Ref. CK.NAC AE1-13) using an ACE® multianalyser (Alfa Wasserman Clinical Chemistry System) and Alfa Wasserman reagents. Serum concentration of non-esterified fatty acid (NEFA) levels were analysed by an ACE® multianalyser (Alfa Wasserman Clinical Chemistry System) with commercial kits (Wako 994-75409 NEFA-C test kit). The concentration of cortisol was determined from plasma (K3-EDTA) by enzyme immunoassay using an "in-house kit" (validated by Chacón et al., 2004). Each sample was determined in duplicate from 50 μl of plasma. The mean of the duplicate was used as the result and expressed in nmol/l. Inter- and intra-assay coefficients of variation were 6 and 9%, respectively. The concentration of lactate was determined using a Sigma Diagnostic kit (lactate no. 735-10) and spectrophotometer (Lambda 5, PerkinElmer).

Eye temperature was taken by infrared thermography (IRT) on reactivity to handling test (Pascual-Alonso et al., 2013). Lambs were randomly captured and restrained by a trained handler. Restraint continued for 1 min. during which a photograph of the left eye (approximate distance 20 cm) was taken with an IR camera (Testo 880 Thermal Imaging Camera; Testo AG, Lenzkirch, Germany) to evaluate acute stress response produced by handling (Stewart et al., 2007; Pascual-Alonso et al., 2013). The built-in lens (24°) was used and the camera was calibrated for room temperature and relative humidity. The emissivity value used was 0.98, which is recommended by the camera manufacturer for biological tissues. A clear infrared image (precise location and perfect focus) was taken of each animal. Image analysis software (IRSoft™ software, Testo AG, Lenzkirch, Germany) was used to determine the maximum temperature within an oval area traced around the eye, including the eyeball and approximately 1 cm around the outside of the eyelids (Stewart et al., 2007).

2.4. Productive performance and meat quality

The amount of concentrate added to the feeder and the feed remaining at the end of the experiment were recorded. Total consumption of concentrate (TCC) was estimated as the difference between the concentrate provided and concentrate remaining in the feeder hopper. Average daily gain (ADG) was calculated by the difference between W2 and W1 (WG) divided by the total fattening period (35 days). The concentrate conversion index (CCI) was estimated as TCC/WG. The animals were slaughtered within the weight range of the Ternasco-type category (Sañudo et al., 1996; Sañudo et al., 1998) at an EU-approved abattoir located in the city of Zaragoza.

After slaughter, carcasses were stored in cold rooms at 2 °C for 24 h. Cold carcasses were weighed (CW) at 24 h (at 1–2 °C) in the cold room. The extent of bruising on the carcasses was estimated visually using an adapted scale of Miranda-de la Lama et al. (2009) with a score of 0 (no bruises), 1 (slight bruising), 2 (moderate bruising) or 3 (high bruising). Carcass conformation score (CS) and carcass fatness (FS) were graded according to the European classification system (EEC regulation, 2137/92 and 461/93), the EUROP conformation scale (converted to a 15-point scale) and the carcass fatness scale (converted to a 15-point scale). After chilling for 24 h the left rack was removed from T1 to L6 vertebrae (standard Spanish lamb cut according to Colomer-Rocher et al., 1988). The pH at 24 h (pH_{ult}) of the M. *longissimus* was assessed using a portable pH metre (fitted with a Crison 52-00 penetration electrode), which was inserted into a small incision in the left loin (L2–L3 vertebrae). The pH metre was re-calibrated after every five samples using two standard buffer solutions, to pH 7.02 and 4.00. After that, the left rack was transferred to the Meat Laboratory at the Faculty of Veterinary Medicine of the University of Zaragoza without breaking the cold chain.

The M. *longissimus* was removed from the rack to prepare the samples. Colour was estimated at 24 h *post mortem* and after 15 min of blooming using a Minolta CM 200 calibrated chromameter with a standard D65 illuminant and a 10° observer with an aperture size of 2.54 cm, following the CIE L*a*b* system, in order to measure the colour of fresh meat on the cut surface of the T13 vertebra of the M. *longissimus*. Chroma (C*) and hue (H*) indices were calculated as $C^* = (a^{*2} + b^{*2})^{0.5}$ (related to the quantity of pigment) and hue $H^* = 1/\tan (b^*/a^*)$ (attribute of colour perception). Final values were the average of three measurements. A section of meat from the T10 to T13 vertebrae was weighed (FMW) (mean 118 g), vacuum-packed, frozen, and stored at −20 °C after 72 h of aging, to evaluate cooking losses (CL%) and perform the Warner–Bratzler test. Samples were thawed for 24 h in a refrigerator (2–4 °C) in their vacuum-sealed plastic bags before testing. The thawed samples were then weighed (TMW) (mean 113.2 g) and cooked for approximately 35 min in plastic bags at 75 °C in a water bath (GLF-D3006), until the internal temperature of the meat (measured with a penetration thermometer) reached 70 °C. They were then cooled for 30 min under cold running water and further cooled to room temperature, after which they were blotted dry using paper towels and weighed (CMW). The CL% was [CL% = 100 − (CMW × 100)/TMW]. The texture of the cooked meat was measured with a Warner–Bratzler device, using an Instron 4301 equipped with a Warner–Bratzler shear. To cut 1 cm^2 pieces (in the direction of the muscle fibres), we used a MITUTOYO digital callipers 500 series (Mitutoyo Corporation, Aurora, IL, USA). Three measurements were taken for each animal. Shear force (kg/cm^2), maximum stress (kg/cm^2), and toughness (kg) were measured as described by Campo et al. (2000). The gauge and the gauge length

Table 2
Behaviours rate (%) observed during scan sampling (1512 scans per treatment) in lambs finished under enriched (double bunk and straw) or control (barren) environment according to the time of day (AM: 8 to 12 h. Noon: 12–16 h. PM: 16–20 h.).

Treatment	Day Moment	Feeding behaviour		
		Feed concentrate	Feed straw	Drinking
Control	AM	11.57 ± 0.65 ax	–	0.52 ± 0.11 ax
	Noon	5.23 ± 0.64 bx	–	0.24 ± 0.11 bx
	PM	6.45 ± 0.63 bx	–	0.34 ± 0.11 abx
Enriched	AM	9.70 ± 0.53 ay	9.21 ± 0.32 a	0.59 ± 0.09 ax
	Noon	4.48 ± 0.52 bx	1.74 ± 0.31 b	0.44 ± 0.09 ax
	PM	4.97 ± 0.52 by	0.77 ± 0.31 c	0.47 ± 0.09 ax
		Standing/resting behaviour		
		Walking	Resting	Standing up
Control	AM	13.71 ± 0.73 ax	58.82 ± 1.36 ax	8.94 ± 0.53 ax
	Noon	2.53 ± 0.72 bx	85.54 ± 1.34 bx	5.48 ± 0.53 bx
	PM	3.43 ± 0.70 bx	83.66 ± 1.31 bx	5.11 ± 0.51 bx
Enriched	AM	11.53 ± 0.60 ay	60.86 ± 1.12 ax	6.84 ± 0.44 ay
	Noon	2.76 ± 0.58 bx	86.23 ± 1.09 bx	3.69 ± 0.43 by
	PM	3.76 ± 0.58 bx	86.19 ± 1.09 bx	3.53 ± 0.42 by

a,b: Different letters represent significant differences between time of day within treatment; x,y: different letters represent significant differences between treatment within time of day AM: morning from 8 to 12 h. Noon: afternoon from 12 to 16 h. PM: evening from 16 to 20 h.

of the sample were 10 mm and 30 mm, respectively. Samples were sheared perpendicularly to the grain. The load cell was 100 kg (minimum load level 0.001 kg), crosshead speed was 150 mm/min (high extension limit = 30 mm), and the sampling rate was 20 points/s.

2.5. Statistical analysis

Data were analysed using SAS/STAT (9.1 SAS Inst. Inc., Cary, NC, USA) by SAS (1998). Production, physiology and meat quality data were analysed using least squares methods of the GLM procedure of SAS (SAS, 1988) fitting a one-way model with a fixed effect of environmental enrichment (two levels). The general representation of the model used was: $y = Xb + e$, where y was an $N \times 1$ vector of records, b denoted the fixed effect in the model with the association matrix X and e was the vector of residual effects. The original full model included the effect of replicate (fitting animals nested within pens), which was found to be non-significant and consequently was dropped from the model. Meat and carcass quality variables were co-varied with cold carcass weight.

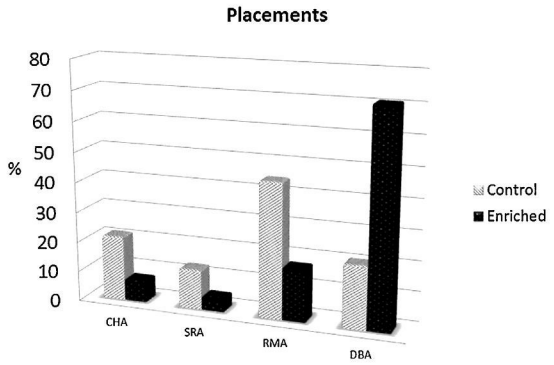
The behaviour data were transformed by the square root function. The social behaviour (average per animal per day) was analysed using PROC MIXED with repeated measurements (day), treatment as the fixed effect and the lamb as the random effect. For maintenance behaviour and stereotypes we added time of day to the model, as the fixed effect. T-maze variables were subjected to repeated-measures ANOVAs that examined the main effects of treatment (control and enriched), T-maze trial (1st and 2nd T-maze escape; the repeated measure), and their interaction. A probability of $P \leq 0.05$ values was considered statistically significant.

3. Results

Overall, the maintenance behaviour pattern and the productive performance traits were similar between treatments, but there were significant differences ($P \leq 0.05$) in the configuration of the use of space, stereotypic behaviours, social interactions, physiological stress and meat quality variables.

3.1. Behaviour and cognitive test

The maintenance behaviour rate of the lambs by time of day is shown in the Table 2. The lambs spent more than three quarters of their time resting. In both treatments significant differences ($P \leq 0.05$) were observed between times of day for this behaviour. As expected, lambs spent significantly less time resting in the morning than during the rest of the day. In the morning lambs fed on concentrate (FC), walked (WK) and stood (ST) more ($P \leq 0.05$) than in the afternoon and evening and there were significant differences between treatments. The CO lambs ate more concentrate, walked more and spent more time standing ($P \leq 0.05$) than ED lambs. FC and WK decreased by the afternoon and evening without any significant differences between treatments, while ST also decreased but remained higher in CO lambs. There was no significant difference in drinking behaviour between treatments. The ED lambs had higher foraging straw (FS) behaviour in the morning ($P \leq 0.05$) than in the afternoon or evening.



CHA= concentrate hopper; SRA= straw rake; RMA= remaining pen; DBA= double bunk

Fig. 3. Occupancy rate (placements) of the lambs in enriched vs. control pens.

The overall placements of lambs in the pen are shown in Fig. 3. The configuration of the use of spaces in pens was significantly different between treatments ($P \leq 0.05$). The use of the DBA was higher in ED lambs than in CO lambs. Most of the lambs using the double bunk (80%) remained on the ground level. CO lambs were observed to use the RMA, CHA and SRA areas more than ED lambs. Placements according to the time of day are shown in Table 3. In both treatments, the occupancy rate of the DBA was lower in the morning ($P \leq 0.05$) than in the afternoon and evening. The use of other areas was greater in the morning in the case of ED lambs ($P \leq 0.05$) than in the afternoon and evening, while the use of CHA

Table 3
Occupancy rate (%) of the different areas in the pen in lambs finished under enriched or barren environments according to the time of day (AM: 8 to 12 h. Noon: 12–16 h. PM: 16–20 h.).

Area	Control pen			Enriched pen		
	AM	Noon	PM	AM	Noon	PM
CHA	23.52 ± 0.72 ax	19.11 ± 0.70 bx	21.80 ± 0.69 cx	10.71 ± 0.59 ay	5.23 ± 0.57 by	5.78 ± 0.57 by
SRA	10.89 ± 0.53 ax	14.91 ± 0.52 bx	13.31 ± 0.51 cx	9.72 ± 0.44 ax	2.34 ± 0.43 by	1.61 ± 0.43 by
RMA	47.24 ± 0.75 ax	43.42 ± 0.74 bx	43.60 ± 0.72 bx	21.82 ± 0.62 ay	15.01 ± 0.61 by	15.68 ± 0.60 by
DBA	18.32 ± 0.97 ax	22.62 ± 0.96 bx	21.37 ± 0.94 bx	57.76 ± 0.80 ay	77.40 ± 0.78 by	76.96 ± 0.78 by
up	–	–	–	15.94 ± 0.58 jt	13.39 ± 0.56 kt	12.34 ± 0.56 kt
dw	–	–	–	41.80 ± 0.93 ju	64.01 ± 0.91 ku	64.61 ± 0.91 ku

H = Concentrate hopper area; SR = straw rake area; DB: double bunk area; RM: rest of the pen. up: upper part of the double bunk. dw: lower part of the double bunk.
Time of day: AM: morning from 8 to 12 h. Afternoon: from 12 to 16 h. PM: evening from 16 to 20 h. a,b,c: different letters represent significant differences between time within treatment.
x,y,z: Different letters represent significant differences between treatment within time of day
j,k,l: Different letters represent significant differences between time of day within area (UP or DW).
t,u,x: Different letters represent significant differences between area (UP or DW) within time of day.

Table 4
Least squares means of the time taken to leave from the isolation chamber (isolation time), the time to resolve the maze (total time) and the number of the areas passed through in the T maze (NQUAD) in the overall task, during the 1st and 2nd exposure to the task in lambs from full enriched or barren (control) environments during the finishing phase of fattening.

Variable	Exposure	Control	Enriched
Isolation chamber	First (s)	18.9 ± 5.09bx	5.8 ± 5.0a
	Second (s)	6.1 ± 5.09y	3.3 ± 5.09
Total time	First (s)	103.27 ± 14.07bx	59.6 ± 14.07a
	Second (s)	50.9 ± 14.07y	45.3 ± 14.07
NQUAD	First	18.06 ± 2.6	15.3 ± 2.6
	Second	12 ± 2.6	15.1 ± 2.6

s): Seconds; a,b: different letters within row represent significant differences between treatments ($P \leq 0.05$); x,y: different letters within column represent significant differences between exposures within treatments ($P \leq 0.05$).

Table 5
Least square means (±S.E.) of physiological stress variables and meat pH in lambs subjected to barren (control) or enriched environments during the finishing phase of fattening.

Response variable	Control	Enriched
Cortisol (nmol/L)	43.88 (±5.58) a	106.24 (±5.58) b
Glucose (mg/dl)	86.80 (±3.16)	85.90 (±3.16)
Lactate (mg/dl)	23.70 (±2.51) a	41.33 (±2.51) b
CK (IU/L)	208 a (±18)	291 b (±18)
NEFA (mg/ml)	0.069 (±0.029) a	0.195 (±0.029) b
Ratio N/L	0.75 (±0.07) a	0.48 (±0.07) b
Haematocrit (%)	27.68 (±0.9)	28.65 (±0.9)
Haemoglobin (g/dl)	10.90 (±0.16)	10.83 (±0.18)
IR Thermography (°C)	37.85 (±0.08) a	38.42 (±0.08) b

Different letters in the same row means significant difference between treatments ($P < 0.05$). N/L: neutrophil/lymphocyte ratio. CK: creatine kinase. NEFA: non-esterified fatty acids. WBC: White blood cells. RBC: Red blood cells. VCM: mean cell volume. IR: infrared thermography. NA: not available.

Table 6
Least square means (±S.E.) of productive performance traits and instrumental meat quality variables in lambs subjected to barren (control) or enriched (double bunk and straw) environments during the finishing phase of fattening.

Response variable	Control	Enriched
Slaughter weight (kg) (W2)	26.30 (±0.38)	25.73 (±0.36)
Average daily gain (g)	263 (±9.34)	257 (±8.86)
Conc. conversion index	3.30 (±0.11)	3.39 (±0.11)
Dressing yield	46.38 (±0.34)	46.04 (±0.33)
Cold carcass weight (kg)	11.88 (±0.21)	11.84 (±0.21)
Bruising score (0–3)	0.13 (±0.08)	0.26 (±0.08)
Carcass conformation score	5.90 (±0.24) a	4.60 (±0.24) b
Carcass fattening score	5.03 (±0.14)	4.90 (±0.14)

Different letters within row represent significant difference between treatments ($P < 0.05$).

and SRA by CO lambs was more erratic and did not follow a clear pattern.

The LS means of stereotypic behaviour performed per day/treatment (continuous sampling) and percentage of lambs performing stereotypes according to the time of day (scan sampling) are shown in Fig. 4a and b, respectively. The CO lambs performed significantly more ($P \leq 0.05$) stereotypic behaviours than ED lambs (Fig. 4a), and the stereotypes in CO lambs were significantly higher during the morning ($P \leq 0.05$) than in the afternoon and evening (Fig. 4b).

Social interactions are shown in Fig. 5(a and b). There were significant differences in aggressive (agonistic) interactions between treatments ($P \leq 0.05$), but not every day (Fig. 5a). The ED lambs had more agonistic interactions than CO lambs ($P \leq 0.05$) on days 2, 4 and 6. On the other hand, the ED lambs were significantly more affiliative ($P \leq 0.05$) than CO lambs during the whole observation period (Fig. 5b). The affiliative interactions of ED lambs increased until day 4, after which the interactions decreased without significant differences on days 2 and 3 of the observation period.

Regarding the T-maze test, significant differences among treatments ($P < 0.05$) were found during the first exposure for time taken to leave to the isolation chamber (IC) and for the total time to solve the T maze. The ED lambs were three times faster leaving the isolation chamber and resolved the maze almost 44 s faster than CO lambs (Table 4). No significant differences were found among treatments in the second exposure to the maze, but the CO lambs significantly reduced the time taken to leave the isolation chamber and the time to solve the maze compared to the first exposure. There were no significant differences between treatments in the overall number of the areas passed through.

3.2. Physiological welfare indicators

The ED lambs had significantly higher levels of cortisol (+142%), lactate (+74.4%), CK (+40%), NEFA (+182%), as well as higher eye IR temperature (+0.57 °C) values than CO lambs (Table 5) while the N/L ratio was higher in CO lambs than ED lambs. There were no significant differences between treatments for glucose levels or for other haematological variables measured.

3.3. Productive performance and meat quality

There were no significant differences in most of the productive traits evaluated (Table 6). There was only a slight difference between treatments for the carcass conformation score (CS), with the CO lambs having a higher conformation ($P \leq$n0.05).

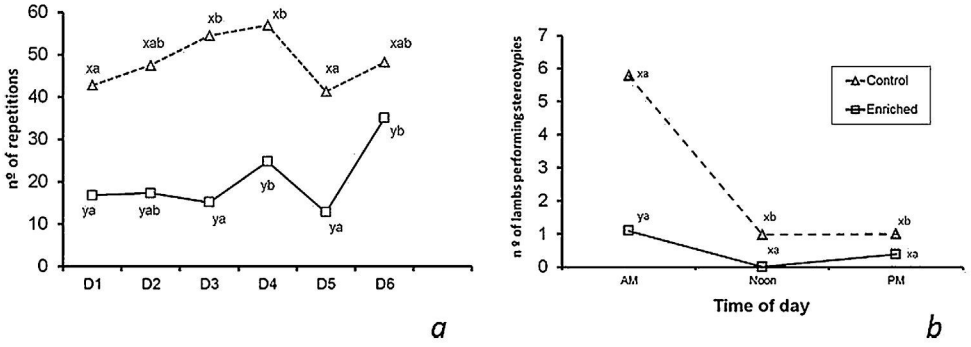


Fig. 4. Least square means of stereotypic behaviours per day/treatment (expressed as average number of repetitions of abnormal behaviour performed by lambs, Fig. 4a) and frequency of lambs performing stereotypic behaviour during the day (average percentage per lamb/treatment according to time of day: AM = 8–12 h, Noon = 12–16 h, PM = 16–20 h, Fig. 4b) and during the 4th week of fattening in lambs finished under enriched or control environments.
Different letters (a,b) represent significant differences between time of day (Fig. 4b) and between days (Fig. 4a) within treatment; different letters (x,y) represent significant differences between treatment within time of day and within day, respectively. The results of Fig. 4a were recorded by continuous sampling. The results of Fig. 4b were recorded by scan sampling.

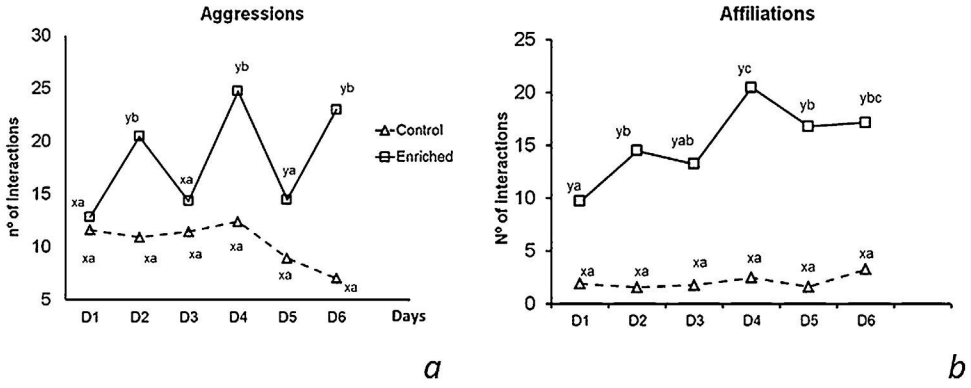


Fig. 5. Least square means of social interactions per day/treatment in lambs given double bunks and straw during the 4th week of fattening compared to lambs without enrichment. Different letters (a, b) represent significant differences ($P \leq 0.05$) between days within the same treatment. Different letters (x, z) represent significant differences ($P \leq 0.05$) between treatments on the same sampling day.

Table 7
Least square means (±S.E.) of instrumental meat quality variables in lambs subjected to control (barren) or enriched (double bunk and straw) environments during the finishing phase of fattening.

Response Variable	Control	Enriched
pH_{ult} (24 h *post mortem*)	5.59 (±0.01)	5.58 (±0.01)
Thawing loses (%)	4.34 (±0.33)a	3.48 (±0.33)b
Cooking loses (%)	14.85 (±0.58)a	12.66 b(±0.58)b
Colour		
L*	42.24 (±0.37)a	40.06 (±0.37)b
a*	19.20 (±0.87)a	16.94 (±0.87)b
b*	7.65 (±0.21)	7.28 (±0.24)
C* (chroma)	20.74 (±0.87)a	18.44 (±0.87)b
H* (hue)	22.49 (± 0.51)	23.21 (±0.51)
Texture by Warner–Bratzler		
Shear force (kg/cm^2)	4.42 (±0.23)a	5.33 (±0.23)b
Toughness (kg)	1.79 (±0.09)a	2.06 (±0.09)b

Different letters within row represent significant difference between treatments ($P < 0.05$).

Regarding instrumental meat quality, there was no difference in pH at 24 h (Table 7). The CO lambs had higher indices of L*, a* and C* (5.4%, 13%, 12.5%, respectively) and ED lambs had higher values of shear force (20.5%) and toughness (15%). Cooking losses (CL%) were higher ($P \leq 0.05$) in CO lambs.

4. Discussion

The behaviour of the lambs kept in enriched housing was more natural, and their social behaviour was complex and richer, with greater and more sustained affiliative interactions and lower levels of stereotypes. Unexpectedly, however, enriched lambs had higher levels of cortisol, which may indicate an acute stress response associated with increased reactivity. The lambs from barren pens presented some signs of immunosuppression.

4.1. Behaviour and the cognitive test

All lambs were more active in the morning, which fits in with the natural pattern behaviour in this species (Gonyou, 1984; Lynch et al., 1992). Unenriched CO lambs spent more time eating concentrate, standing up and had more stereotypes, which may be due to the lack of straw (redirecting of unsatisfied foraging behaviour) as also found by Cooper and Jackson (1996) and Teixeira et al. (2014). Likewise, enriched lambs that had access to straw spent more time foraging (and also ruminating and exploring), especially in the morning after receiving fresh forage (more attractive odour). The results observed in our study generally agree with those described by Teixeira et al. (2014) in the same type of lambs under similar production conditions. The presence of the double bunk did not significantly alter resting behaviour, corroborating previous findings that resting patterns are inelastic and difficult to modify by environmental conditions (Jørgensen et al., 2009), especially in young, almost pre-ruminant lambs.

The lambs in our study mostly fed in the morning, although in extensive systems sheep tend to graze throughout the day (Broom and Fraser, 2010). In intensive indoor conditions (as in our study), the high energy value of food (generally offered *ad libitum*) allows lambs to easily satisfy their nutritional requirements, which will

finally influence their feeding behaviour (Rutter, 2000). The presence of straw can lead them to have a more natural behavioural repertory in that it keeps them busy longer and distracted from performing abnormal behaviours. Straw is thus a source of fibre but also a source of sensory stimulation, which motivates lambs to forage and ruminate more, and also to explore (Teixeira et al., 2014). The behavioural repertory of CO lambs was thwarted due to the absence of straw (as forage or bedding), which probably reduces their welfare and affects the normal ontogeny of relevant behaviours in this species (Pearce and Paterson, 1993; Tuyttens, 2005). Mornings could be a critical time for the development of abnormal behaviours in feedlot lambs, since stereotypes are used as an indicator of lack of welfare as they provide information about the level of stress (Mason, 1991; Miranda-de la Lama et al., 2012). This should be considered when making decisions to establish enrichment programs to be applied under intensive feedlot systems.

With regards to placement, the ED lambs spent more time on the DB, suggesting a strong preference, especially during the afternoon and evening, when most of the lambs rested. Hansen and Lind (2008) studied 6, 11 and 18-month-old lambs and found that only the 6-month-olds preferred the ground level (only a tendency) of the double bunk where the headroom was at 60 cm. The DB height in our study was only 50 cm but the lambs were younger (3 months old) and smaller. Therefore, age seems to have an influence on DB use, especially the ground level. One reason may be that younger lambs are more fearful and prefer to be sheltered. However, that same "shelter effect" would reduce visual contact with the outside environment and handlers, and appears to elicit an increase in reactivity to handling (e.g blood sampling). Another possible reason could be related to the genetic differences of Mediterranean breeds in comparison to northern European breeds, which are less gregarious and not subjected to a sheepherding system.

With regard to social interactions, enrichment encouraged sociability, since it was associated with an increase in affiliative interactions and sporadic peaks of aggressive behaviour without disrupting social stability (Weary et al., 2008). In a previous study using the same type of lambs, a lack of straw increased social affiliations (Teixeira et al., 2014), but that was explained by the more barren physical environment. In the present study however, the enriched environment increased proximity among lambs, which is most probably the main reason for the higher levels of affiliative behaviour. Affiliative behaviour can be used as an indicator of positive experiences and group cohesion in farm animals under commercial conditions (Boissy et al., 2007). From the results obtained we would suggest that the proposed full enrichment set had a positive effect on reducing abnormal behaviours, increasing the behavioural repertoire, and encouraging social interactions that promote group stability in this type of lambs and productive system.

A T-maze test can be used to assess the detrimental effects of stressful conditions provoked by multifactorial effects of housing and handling during fattening (Hutchinson et al., 2012). The ED lambs performed better than CO lambs on the first trial but results were similar after the second exposure, which may indicate some type of compensatory learning in the CO lambs. The results obtained suggest that the ED lambs responded more efficiently to the novelty of the T-maze during the first exposure in comparison to the CO lambs, but their learning capacity did not differ significantly from the control lambs.

4.2. Physiological welfare indicators

As previously mentioned, the HPA activity of ED lambs was higher than that of CO lambs, as has also been found in goats provided with enrichments using elevated areas (Miranda-de la Lama et al., 2013). Our lambs were able to use the enrichment in different ways, one of these being to shelter on the ground level of the DB. This shelter position reduces visual contact with the surrounding environment, which probably provoked a greater response to handling procedures like blood sampling. This was also supported by the higher eye (IR) temperature observed in the reactivity to handling test. Dhabhar (2002) and Dhabhar and McEwen (1997) propose that, under conditions involving acute stress, both innate as well as adaptive immune-responses may be significantly enhanced, as occurred in our study. They suggest that the physiological stress response may play a critical evolutionary adaptive role that prepares the immune system (redistribution of monocytes and lymphocytes to the skin and lymph nodes) for potential challenges (i.e wounds or infections). Hence, it is a field for further investigation, especially in lambs that spend longer periods of fattening time in intensive housing systems.

Moreover, the acute response of the ED lambs could also be associated with their high energy expenditure and muscular activity, indicated by elevated lactate, NEFAs and CK. Plasma CK is generally considered to be a chemical indicator of physiological stress, muscular damage and fatigue (Miranda-de la Lama et al., 2012). In our case, the higher CK in ED lambs could be due to intense physical stress in sensitive lambs during handling, although, on the other hand, Kaneko et al. (2008) describe that higher levels of CK could also be caused by prolonged recumbence, which was also the case in our study.

The higher level of N/L ratio ($P < 0.05$) in CO lambs suggest some signs of chronic stress, probably due to barren housing conditions. The N/L ratio is a measure widely used to assess chronic stress (Davis et al., 2008; Dhabhar, 2002). Stress-induced reductions in circulating lymphocytes are caused mainly because glucocorticoids induce alterations in the trafficking or redistribution of lymphocytes from the blood to other body compartments (Dhabhar, 2002). However, our results would corroborate only a part of this fact, since the range of cortisol values in CO lambs was normal for this species. Thus, it seems that some other effect caused immunosuppression in CO lambs, possibly related to the barren environment that provided low sensory stimulation and poor welfare.

4.3. Performance and meat quality

The performance of ED lambs was similar to CO lambs, with the exception of ED lambs having a lower carcass conformation. However, there were no differences in fatness, which demonstrates the difficultly of assessing carcass quality in very light lambs and the need to develop a specific European scale to assess light lamb carcasses produced in the Mediterranean area. Ultimate pH is the main indicator of meat quality at a commercial level since it can affect important quality characteristics such as cooking loss, colour and texture (Miller, 2002). Ultimate pH values were similar among treatments and within the range for commercially acceptable meats, but enrichment had certain effects on cooking loss, colour and texture that are not easy to explain. One possible reason for the variation in colour could be the level of physical activity in an indoor environment (Dunne et al., 2011). Although ED lambs spent most of their time resting, they also climbed and played around the furniture, which may have had a particular effect on back muscles (i.e *longissimus dorsi*) producing slightly darker and tougher meat. Exercised young bulls have a relative increase in slow-contracting fibres, a better vascularization, higher oxidative metabolic potential and darker meat colour compared with young bulls in intensively-fed tie-stalls (Vestergaard et al., 2000). The muscle fibres of the ED lambs may have changed in response to physical activity (Vestergaard et al., 2000). However, the lower redness (a*) values of ED lambs are more difficult to explain and could be related to lower cooking losses and higher toughness

of the meat, also lowering the carcass conformation score. The muscle fibres of exercised animals are larger in relation to the tougher collagen component, which could explain the tenderness of the meat (Aalhus et al., 1991; Gregory, 1998). However, since ED lambs spent most of their time resting (under the double bunk), this may have reduced the muscle fibre to collagen component ratio.

5. Conclusion

The full enrichment used had a positive effect on lamb behaviour, favouring a richer behavioural repertoire, reducing stereotypes and increasing affiliative interactions, which improves group cohesion. Enrichment also improved immunity and had no detrimental effects on performance or commercial meat quality; although further studies related to muscle fibre composition would be necessary to deepen in the causes of tougher meat. It is important to remark here that sometimes a good enrichment system that takes into account the species' natural behaviour may not be the most efficient way to manage animals, as this may make handling more difficult at the feedlot and become in a less efficient way to produce and manage stabled animals. Therefore, it recommend that enrichment items should be carefully studied taking account not only the behaviour of lambs but something that facilitates their handling at stabled conditions in order to avoid consequences that may jeopardize the ultimate efficiency of the system.

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